Table 2. Mini-sequencing results (as measured by optical density, OD_{450}) obtained from digoxigenin-labeled dATP or dUTP reactions with amplified genomic DNA templates containing the [S291G] point mutation site of the AChE gene from SS and AZ-R strains of Colorado potato beetle

CPB	OD_{dATP}	$OD_{dATP} - OD_{ddH2O}$	Mean (±SD)	OD_{dUTP}	$OD_{dUTP} - OD_{ddH2O}$	Mean (±SD)
SS1	0.407	0.304		0.174	0.115	
SS2	0.434	0.331		0.104	0.045	
SS3	0.49	0.387		0.137	0.078	
SS4	0.434	0.331	0.338 (±0.035)	0.125	0.066	0.076 (±0.029)
AZ-R1	0.155	0.052		0.127	0.068	
AZ-R2	0.116	0.013		0.112	0.053	
AZ-R3	0.123	0.02	0.028 (±0.021)[b]	0.148	0.089	0.070 (±0.018)
ddH_2O[a]	0.103			0.059		
SS5	0.39	0.292		0.125	0.076	
SS6	0.369	0.271		0.142	0.093	
SS7	0.402	0.304		0.156	0.107	
SS8	0.425	0.327	0.301 (±0.023)	0.153	0.104	0.095 (±0.014)
AZ-R4	0.14	0.042		0.101	0.052	
AZ-R5	0.148	0.05		0.182	0.133	
AZ-R6	0.115	0.017	0.036 (±0.017)[b]	0.154	0.105	0.097 (±0.041)
ddH_2O[a]	0.098			0.049		

[a] Double-distilled water was added instead of AZ-R genomic DNA template as control.
[b] Mean value was significantly different from SS mean value ($P < 0.001$).

results in an increased OD (Table 2). However, the presence of the resistant GGT allele can be directly determined using cPASA as previously described by Zhu and Clark.[7]

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Ligands of the nicotinic acetylcholine receptor as insecticides

Ralf Nauen,* Ulrich Ebbinghaus and Klaus Tietjen
Bayer AG, Agrochemicals Division, Research, D-51368 Leverkusen, Germany

Abstract: Insect nicotinic acetyl receptors (nAChR) are targets of growing importance and, since the early 1990s, the number of such highly effective insecticides as imidacloprid and spinosyn has grown. Several natural compounds, eg dihydro-β-erythroidine, methyl caconitine and paraherquamide, showing high affinity to the same receptor, were considerably less active as insecticides, most likely because of their antagonistic action. Our observations on aphids after ingestion of the antagonistic compound dihydro-β-erythroidine revealed anti-feedant-like properties. As a consequence, the symptomology of poisoning was totally different between agonists and antagonists of the nAChR. Electrophysiological (whole-cell voltage clamp) measurements in isolated housefly neurones revealed that agonism seems to be a prerequisite for insecticidal activity. Furthermore, we were able to demonstrate the existence of two different subtypes of the nAChR in isolated locust neurones with different pharmacology and ion-channel properties.

Keywords: Imidacloprid; neonicotinoids; chloronicotinyls; dihydro-β-erythroidine; methyl caconitine; paraherquamide; nicotinic acetylcholine receptor; *Myzus persicae*; electrophysiology; antifeedant; *Locusta migratoria*; *Musca domestica*

Background

Only ten years ago insecticides acting on the nicotinic acetylcholine receptor (nAChR) were of minor economic importance (<2% of the total insecticide market in 1991) and registered compounds included cartap (1964), thiocyclam (1977) and bensultap (1968) which were metabolised to nereistoxin, a naturally occurring toxin described in the marine worm *Lumbriconereis heteropoda* Marenz, within the insect's body. Nicotine is one of the oldest known insecticides and the compound is still used to control some homopteran pests in greenhouses.

A totally different class of compounds which affect nAChR with considerable biological efficacy against key homopteran and coleopteran pests are the chloronicotinyls or neonicotinoids,[1] a new chemical class of insecticide introduced to the market in the early 1990s.

* Correspondence to: Ralf Nauen, Bayer AG, Agrochemicals Division, Research, D-51368 Leverkusen, Germany
E-mail: ralf.nauen.rn@bayer-ag.de

(*Received 13 October 1998; accepted 5 January 1999*)

Ligand	AChR-Affinity pI_{50}[a]	Agonism (Ach = 1)[b]	Biological efficacy[c]
Dihydro-β-erythroidine	8.2	0	1
Methyl caconitine	9.0	0	0.5
Paraherquamide	9.3	0	0
Imidacloprid	9.1	0.74	4.1
Acetamiprid	8.7	0.92	3.7
Nitenpyram	8.6	2.17	3.6

[a] [^{3}H]Imidacloprid binding site in head homogenates of *Musca domestica* (pI_{50} = −Log M).
[b] Revealed by electrophysiological whole-cell recording on neurones from *Musca domestica*.
[c] Potency against *Myzus persicae* in oral ingestion bioassays (arbitrary units).

Table 1. Characteristics of insecticidal and non-insecticidal ligands of insect nicotinic acetylcholine receptors

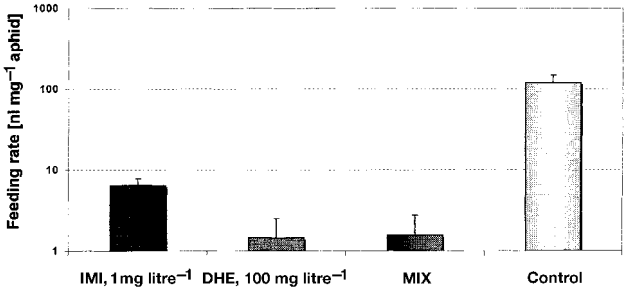

Figure 1. Influence of imidacloprid (IMI) and dihydro-β-erythroidine (DHE) on diet up-take of *Myzus persicae* feeding on artificial double membranes assessed after 4 h.

Imidacloprid vs Spinosyn

The first commercial compound belonging to this promising group was imidacloprid (trade names: Confidor, Gaucho, Admire, Provado), an agonist of the nAChR.[2] Other compounds, belonging to the same chemical class are likely to be launched within the next few years. The insecticide market share of the chloronicotinyls (or neonicotinoids) is predicted to rise from <2% in 1991 to some 10–15% by the beginning of the next millennium.[3] The nAChR is likely to become the most important target after acetylcholinesterase and the voltage-gated sodium channel.

The spectrum of activity of spinosyn, (XDE-105; BSI-proposed common name, spinosad) a macrolide mixture from *Saccharopolyspora spinosa*, shows that it is not only homopterans and coleopterans that are controlled by compounds affecting the nAChR. This new material is not effective against homopterans, but is particularly active against noctuid larvae. Physiological observations by Salgado[4] suggested that spinosyn acts differently from imidacloprid. Electrophysiological data revealed that spinosyn as well as the neonicotinoids, acts as an agonist of nAChR.[5] Additional electrophysiological measurements carried out by Dunbar *et al.*[6] suggested that the site of action of spinosyn on nAChR is distinct from the acetylcholine binding site.

Agonists vs Antagonists

Receptor binding studies using [^{3}H]imidacloprid as a radioligand in homogenates of aphids, whiteflies, cat fleas and houseflies revealed comparable affinity of chloronicotinyls to their nAChRs. Several natural compounds acting on the same target, eg dihydro-β-erythroidin, methyl caconitine and paraherquamide, show a considerably lower potential as insecticides, though their binding affinities to housefly receptors do not differ widely from those of the chloronicotinyls. Electrophysiological measurements in isolated housefly neurones revealed that compounds acting agonistically on the nAChR were in general insecticidal, whereas antagonistic compounds were mostly non-active, as shown by the lack of symptoms of poisoning typical for compounds interfering with the insect nervous system (Table 1). Some antagonists, such as dihydro-β-erythroidine, alter the behaviour of aphids, eg *Myzus persicae* Sulz and show clear anti-feedant effects in oral ingestion bioassays, (Fig 1) as measured while feeding on radio-labelled diet. Mortality observed in such short-term artificial feeding experiments (4 h) was high for imidacloprid alone (>50%), low for dihydro-β-erythroidine (4%) and also quite low for a mixture of the two (16%), suggesting that dihydro-β-erythroidine prevents the agonistic effects of imidacloprid necessary for irreversible symptoms of poisoning in *M persicae*. It remains open whether the antifeedant-like properties of imidacloprid recently reported for aphids and whiteflies could be attributed to its partially antagonistic behaviour.[7,8] Also still to be clarified is whether the different physiological observations regarding the symptomology of poisoning can be attributed to different subtypes of nAChRs. Our electrophysiological studies (whole-cell voltage clamp method) performed on isolated neurone cell bodies isolated from adult locust head ganglia resulted in the description of the functional expression of two different nAChR subtypes, at least in locust neurones.

Type A vs Type B Currents

On isolated locust neurons, acetylcholine (ACh) at saturating concentrations (1000 μM) induced a fast inward current with an amplitude of up to 7 nA. The time course of the response was quite variable from one cell to another. In addition to the rapidly desensitizing component present in most cells, some

cells also exhibited a non-desensitizing response to ACh.

On cells which had predominantly the fast desensitizing component (Type A, >90%), only ACh acted as a full agonist, whereas anatoxin A, nicotine, cytisine and the insecticide imidacloprid were all partial agonists. The agonist efficacy rank order was ACh ≫ anatoxin A ≫ imidacloprid = nicotine. Cytisine was the least effective agonist on these cells, eliciting a maximal response about 10 times lower than that observed for 1000 μM ACh. Anatoxin A was the most potent agonist, with an EC_{50} of 0.4 μM. On the same cell, ACh exhibited an EC_{50} of 13.5 μM. In contrast to imidacloprid ($EC_{50} \sim 1$ μM), nicotine had a relatively low potency, with an EC_{50} of 29 μM. These results gave a rank order of potency of anatoxin A > imidacloprid > ACh > nicotine for this component. On cells which had no fast-desensitizing component (Type B) ACh, anatoxin A, cytisine, nicotine and imidacloprid elicited nearly the same maximal responses at saturating concentrations. Anatoxin A was again the most potent agonist, with an $EC_{50} < 0.1$ μM. On the same cell, nicotine and cytisine exhibited practically the same EC_{50} of ~0.5 μM. ACh had the lowest potency with an EC_{50} of ~5 μM, and imidacloprid showed an EC_{50} of 3 μM. Taken together, these results gave a rank order of potency of anatoxin A > nicotine = cytisine > imidacloprid > ACh. With the exception of imidacloprid, the EC_{50} values for all agonists tested were lower than the corresponding EC_{50} values obtained from cells with mostly rapidly desensitizing ACh-induced currents. However, the most remarkable pharmacological difference between cells in which one or the other type of ACh response predominated is the clearly distinct affinity for nicotine, which can differ by a factor of 100.

In summary, our electrophysiological data confirm the existence of functional nAChR in locust neurones with different ion-channel properties and distinct affinity for ligands, suggesting that there exist (at least) two different and independent populations of nicotinic receptors in the insect nervous system. The same conclusions have been derived very recently by van den Beukel *et al*[9] when measuring the differential activation of nAChR subpopulations in *Locusta* neurones using physostigmine and acetylcholine.

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Chemistry, stereochemistry and biological properties of KWG 4168

Wolfgang Krämer,* Dieter Berg, Stefan Dutzmann, Wilfried A Etzel, Wolfgang Gau, Ulrich Stelzer and Joachim Weissmüller

Bayer AG, Crop Protection Business Group, Agricultural Centre Monheim, D-51368 Leverkusen, Germany

Abstract: KWG 4168 (8-*tert*-butyl-1,4-dioxaspiro[4,5]decan-2-ylmethyl(ethyl)(propyl)amine; proposed common name spiroxamine) is a new fungicide consisting of four biologically active isomers (two diastereomers, four enantiomers). The four isomers were separated by preparative HPLC on a chiral stationary phase. The diastereoisomers were synthesised from the corresponding chirally pure glycerol derivatives and were separated by preparative HPLC. COSY, HSQC and NOESY NMR spectroscopy were used to asign the configuration of the amino residue relative to the cyclohexyl ring. Studies of the activity against wheat powdery mildew, as well as the inhibition of sterol biosynthesis in fungi by the four stereoisomers, indicate the contribution of each isomer to the biological activity of spiroxamine.

Keywords: KWG 4168; spiroxamine; fungicide; biological activity; contribution of each isomer

1 INTRODUCTION

1,3-Dioxolan-4-methylamines, with the general structure **1** (Fig 1) have been synthesised as potential cereal fungicides within our studies on sterol biosynthesis inhibitors.[1] Coupling the residues R^1 and R^2 into a carbocyclic ring to produce spirocycles would be expected to reduce the number of the conformers, producing a better fit at the receptors in sterol biosynthesis. The spiroketalamine class of substances thus produced[2] has the general structure **2**. The first representatives of this class, 1-oxa- and 1,4-dioxa-

* Correspondence to: Wolfgang Krämer, Bayer AG, Crop Protection Business Group, Agricultural Centre Monheim, D-51368 Leverkusen, Germany